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5-Aryl-pyrazolo[3,4-*b*]pyridazines: Potent Inhibitors of Glycogen Synthase Kinase-3 (GSK-3)

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Abstract—Introduction of a nitrogen atom into the 6-position of a series of pyrazolo[3,4-*b*]pyridines led to a dramatic improvement in the potency of GSK-3 inhibition. Rationalisation of the binding mode suggested participation of a putative structural water molecule, which was subsequently confirmed by X-ray crystallography.
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Glycogen synthase kinase-3 (GSK-3) is a serine/threonine kinase implicated in the control of several regulatory proteins.[1] It was first discovered by virtue of its ability to phosphorylate and inactivate glycogen synthase, the regulatory enzyme of mammalian glycogen synthesis. Since then a number of other substrates have been identified, implicating GSK-3 in the regulation of several physiological processes. Recently we identified pyridazine 1 through pharmacophore searching of the SmithKline Beecham in house database.[2] Simplification of pyridazine **1** and subsequent optimisation afforded a potent series of pyrazolo[3,4-*b*]pyridines such as **2** (Fig. 1).

Previously we had shown, from initial SAR studies, that removal of nitrogen at the 6-position of the pyridazine **1** was well tolerated, however, removal of the nitrogen at the 7-position had detrimental effects on GSK-3 potency. Having identified the pyrazolo[3,4-*b*]pyridines as potent inhibitors of GSK-3, we decided to reinvestigate the SAR around the pyridine nucleus (Table 1).

In this series removal of the nitrogen at position 7 (cf. **3**, **4**) was well tolerated. Interestingly, reintroduction of the nitrogen at N-6 (cf. **3**, **5**, **6**) afforded a dramatic improvement in potency. Having established that potency could be improved through this modification we decided to acylate our original lead **1** to afford **8**. Unfortunately, this led to a reduction in potency, which could be consistent with a steric clash between the required conformation of the amide bond and the substituent at C-4. Further evidence supporting this rationale is the finding that the pyrimidine **7** is also a

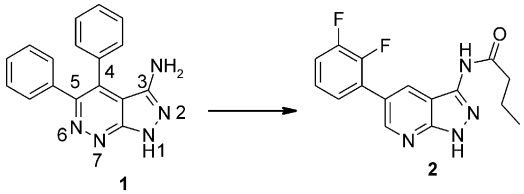
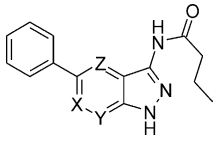


Figure 1. Optimisation of pyridazine 1.

Table 1. Inhibition of hGSK-3-α by selected analogues



No.	X	Y	Z	GSK-3α, IC_{50} nM[3]
3	CH	N	CH	56±6
4	CH	CH	CH	99±15
5	N	N	CH	4±1
6	N	CH	CH	7±1
7	N	CH	N	2697
8	N	N	CPh	691±15

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weak inhibitor of GSK-3 suggesting a lone pair repulsion between the nitrogen at position 4 and the carbonyl of the amide bond. Docking studies with either **5** or **6** into a GSK-3α homology model, (constructed using the crystal structure of the highly homologous cyclin dependant kinase-2 (1 hcl)), failed to provide a rationale for the dramatic increase in potency observed for introduction of nitrogen at the 6-position. In order to explain this affect we turned to the reported X-ray structure of a series of 2-aminothiazoles co-crystallised with cyclin dependant kinase-2 (CDK-2).[4] Interestingly the 2-aminothiazole nucleus forms a similar three point hydrogen bond with the hinge region of the ATP binding site but, perhaps more importantly, one of the ketone lone pairs is involved in a hydrogen bond to a structural water molecule.[5] An overlay of the 2-aminothiazole with the proposed binding mode of **5** in the GSK-3 homology model suggested that the increase in potency may be due to the presence of a structural water molecule in GSK-3 which is not present in our homology model (Fig. 2). Indeed 2-aminothiazoles have recently been disclosed as potent GSK-3 inhibitors.[6] Gratifyingly, this binding hypothesis has recently been confirmed with the determination of a co-crystal structure of inhibitor **9** bound to GSK-3 (Fig. 3).

Having identified the pyrazolopyridazine **5** as a potent inhibitor of GSK-3 we sought to increase water solubility utilising the amide moiety as this is orientated out of the active site towards solvent. Previously, in the related pyrazolopyridine series, we had demonstrated that a butyramide group at position 3 was of optimal length for GSK-3 potency so we decided to explore incorporation of basic amine groups onto this side chain. Importantly, attachment of a basic amine group on to the butyramide side chain was tolerated, albeit with a ca. 5-fold loss in potency (cf. **5**, **9**) (Table 2). A number of heterocyclic amines were also prepared in order to probe for hydrogen bonding interactions, however only modest improvements in potency were observed. Increasing the chain length had no benefits in terms of potency (cf. **11**, **14**). The modest improvement in potency observed when cyclising the butyramide side chain to afford the piperidine analogue **13** was disappointing given the increase in potency we observed for cyclic aliphatic side chains in the structurally similar pyrazolopyridine series.[2] Previously we had identified the 2,3-diF-Ph moiety as an optimal C-5 group in the structurally related pyrazolopyridine series. Gratifyingly, incorporation of this C-5 group into the pyridazine series afforded a similar increase in GSK-3 potency to that observed previously (cf. **9**, **15**).

Having identified a potent series of water-soluble GSK-3 inhibitors, a selection were profiled against a panel of more than 20 kinases including GSK-3β (Table 3).[7] Interestingly incorporation of a basic side chain appeared to give an excellent improvement in selectivity against the highly homologous CDK-2 and to ascertain the degree of CDK-2 selectivity, cK_i's were obtained for a small subset of inhibitors. It is clearly evident from Table 4 that an excellent improvement in selectivity is obtained. From an overlay of the GSK-3 and CDK-2 crystal structures it appears that the origin of this selec-

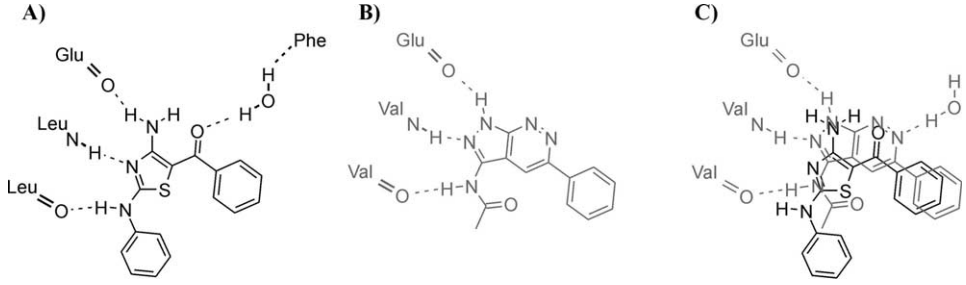


Figure 2. (A) X-ray of 2-aminothiazoles in CDK-2; (B) model of pyrazolopyridazines in GSK-3; (C) overlay of A and B.

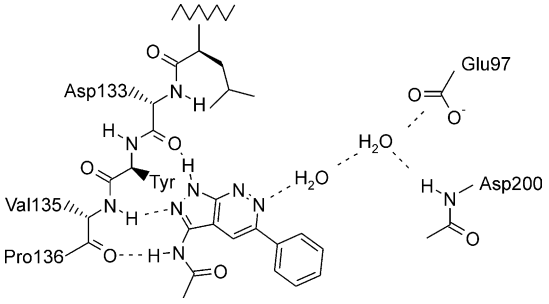
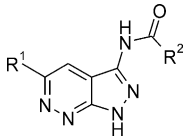


Figure 3. X-ray co-crystallisation of pyrazolo[3,4-*b*]pyridazines with GSK-3.

Table 2. Inhibition of hGSK-3-α by pyrazolopyridazines containing basic side chains



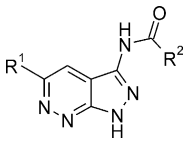
No.	R^1	R^2	GSK-3α, IC_{50} nM[3]
5	Ph	Bu	4±1
9	Ph	$(CH_2)_3NMe_2$	22±2
10	Ph	$(CH_2)_3$pyrrolidine	11±1
11	Ph	$(CH_2)_3$piperazinyl-N-Et	7±2
12	Ph	$(CH_2)_3$morpholinyl	5±1
13	Ph	4-Piperidine-N-Me	9±1
14	Ph	$(CH_2)_4$piperazinyl-N-Et	5±1
15	2,3-diF-Ph	$(CH_2)_3NMe_2$	5±1

Table 3. Selectivity of pyridazine 5 and 9 for GSK-3β inhibition[a]

No.	AMPK	Chk1	CKII	JNK	LCK	MAPK	RSK-2	MAPKAP-K2	MEK1	MSK1	P70S6K	PDK1	PHOS.K	PKA	PKBa	PKCA	PRAK	ROKa	SAPK2a	SAPK2b	SAPK3	SAPK4	SGK	CDK2/Cyclin A	GSK-3β
5	12	0	18	6	0	15	0	0	5	0	0	0	0	0	14	0	21	5	0	5	0	0	0	90	99
9	18	25	16	4	35	5	11	0	9	16	2	8	30	8	0	5	7	14	12	11	0	0	12	45	93

[a]Values are %I @10 μM using 100 μM ATP (see ref 7 for kinases used and assay details).

Table 4. CDK-2 selectivity determination

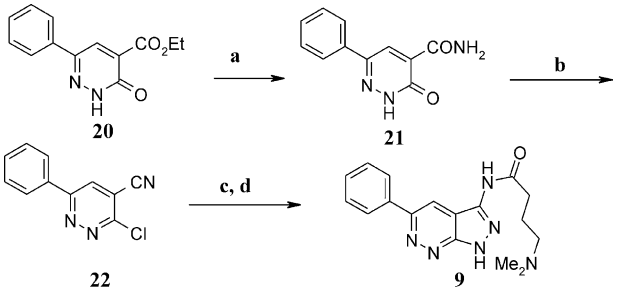


No.	R^1	R^2	GSK-3α, cK_i (nM)	CDK-2, cK_i (nM)	CDK-2/GSK-3
16	3-Pyridyl	cyPr	0.08	5	62
17	2,3-diF-Ph	cyPent	0.11	5	45
9	Ph	$(CH_2)_3NMe_2$	2.5	1695	678
18	2,3-diF-Ph	Piperidine-N-Et	0.95	450	474
19	2,3-diF-Ph	CH_2Piperidine-N-Et	0.19	540	2842

tivity is likely to be due to a steric/electronic clash with the basic group of R^2 and a salt bridge, which is present in CDK-2 but not in GSK-3.

Chemistry[8,9]

The pyrazolopyridazine analogue **9** was prepared as outlined in Scheme 1. Treatment of the commercially available ester **20** with aqueous ammonia at room temperature afforded the amide **21**. Subsequent treatment with phosphorus oxychloride at reflux afforded the chloronitrile **22**. Cyclisation utilising hydrazine hydrate and subsequent selective acylation of the primary amine afforded the desired pyrazolopyridazine **9** in excellent overall yield.

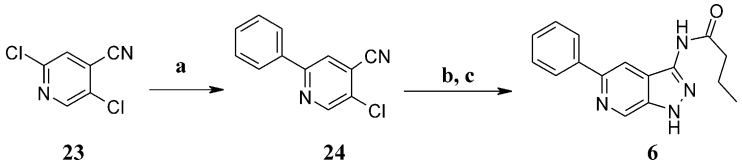


Scheme 1. Preparation of pyrazolopyridazine **9**. Reagent and conditions: (a) NH_4OH (77%); (b) $POCl_3$, reflux, 3 h (100%); (c) $N_2H_2{\cdot}H_2O$, EtOH, reflux, 12 h (100%); (d) $ClCO(CH_2)_3NMe_2$, pyridine, reflux, 2 h (80%).

Preparation of the pyrazolo[3,4-*c*]pyridine **6** is outlined in Scheme 2. Selective Suzuki cross-coupling of phenyl boronic acid with the known dichloronitrile **23**[10] afforded the chloronitrile **24**. Subsequent cyclisation utilising hydrazine hydrate and selective acylation of the resulting amine afforded the desired pyrazolo[3,4-*c*]pyridine **6** in good overall yield.

Summary

Introduction of a nitrogen atom into the 6-position of a series of pyrazolo[3,4-*b*]pyridines led to a dramatic improvement in the potency of GSK-3 inhibition. Rationalisation of the binding mode suggested participation of a putative structural water molecule, which was subsequently confirmed by X-ray crystallography. Introduction of tertiary amine side chains at the C-3 position afforded inhibitors with excellent GSK-3 potency (IC_{50}'s < 22 nM), improved CDK-2 selectivity and good aqueous solubility (> 1 mg/mL).



Scheme 2. Preparation of pyrazolo[3,4-*c*]pyridine **6**. Reagent and conditions: (a) phenylboronic acid, Na_2CO_3, DME, H_2O, EtOH, reflux (60%); (b) $N_2H_2{\cdot}H_2O$, EtOH, reflux, 12 h (59%); (c) butyryl chloride, pyridine, reflux (90%).

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